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Health and Environmental Effects Profile for Trimethyl Phosphate

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16. ABSTRACT

The Health and Environmental Effects Profile for trimethyl phosphate was prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA) and to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files were evaluated as they pertained to potential human health, aquatic life and environmental effects of hazardous waste constituents.

Quantitative estimates have been presented provided sufficient data are available. Trimethyl phosphate has been evaluated as a carcinogen. The human carcinogen potency factor (q_1^*) for trimethyl phosphate is $3/83 \times 10^{-2}$ $(mg/kg/day)^{-1}$ for oral exposure.

The Reportable Quantity (RQ) value of 1, 10, 100, 1000 or 5000 pounds is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified by CERCLA based on chronic toxicity. The RQ value for trimethyl phosphate is 100.

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EPA/600/X-85/367
September 1985

HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR TRIMETHYL PHOSPHATE

ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
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PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste and Emergency Response by the Office of Health and Environmental Assessment. The HEEPs are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA), as well as to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched." The literature search material is current through November, 1984.

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Acceptable Daily Intakes (ADIs) for chronic exposures. An ADI is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, ADIs are not estimated in this document series. Instead, a carcinogenic potency factor of q_1^* (U.S. EPA, 1980) is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under CERCLA. These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity and acute mammalian toxicity).

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEPs will become part of the EPA RCRA and CERCLA dockets.

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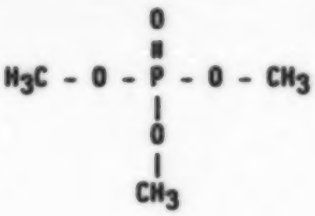
LIST OF ABBREVIATIONS

BCF	Bioconcentration factor
CAS	Chemical abstract service
CS	Composite score
DNA	Neoxyribonucleic acid
GI	Gastrointestinal
i.p.	Intraperitoneal
K_{oc}	Soil sorption coefficient
K_{ow}	Octanol/water partition coefficient
LD_{50}	Dose lethal to 50% of recipients
LOAEL	Lowest-observed-adverse-effect level
MED	Minimum effective dose
MNCV	Maximum nerve concentration velocity
MTD	Maximum tolerated dose
NOAEL	No-observed-adverse-effect level
RQ	Reportable quantity
RV_d	Dose-rating value
RV_e	Effect-rating value
SGOT	Serum glutamate oxaloacetic transaminase
SGPT	Serum glutamate pyruvic transaminase
TWA	Time-weighted average

1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

Trimethyl phosphate is the common chemical name of this compound, but it is also known by the synonyms methyl phosphate, trimethyl orthophosphate, trimethyl ester phosphoric acid, TMP and trimethoxyphosphine oxide. The structure, molecular weight, empirical formula and CAS Registry number for trimethyl phosphate are as follows:



The molecular weight of trimethyl phosphate is 140.08 and the empirical formula is $C_3H_9O_4P$. The CAS Registry number for this compound is 512-56-1.

1.2. PHYSICAL AND CHEMICAL PROPERTIES

Trimethyl phosphate is a colorless liquid that contains 22.1% phosphorus, and it is soluble in both gasoline and water (Hawley, 1977). The available data regarding the physical properties of trimethyl phosphate are listed below:

Boiling point:	193°C	Hawley, 1977
Pour point:	-46°C	Hawley, 1977
Density (68°F):	1.210 g/mL	Hawley, 1977
Refractive index (20°C):	1.397	Hawley, 1977
Flash point:	>300°F	Hawley, 1977
Water solubility:	soluble very soluble	Hawley, 1977 Weast, 1966

Vapor pressure:		
at 26°C	1 mm Hg	Perry and Green, 1984
at 53.7°C	5 mm Hg	Perry and Green, 1984
at 67.8°C	10 mm Hg	Perry and Green, 1984
at 20°C	0.7 mm Hg (extrapolated)	Perry and Green, 1984
at 10°C	0.35 mm Hg (extrapolated)	Perry and Green, 1984
Log K_{OW}:	-0.52 to -0.78	Hansch and Leo, 1981
pKa:	15.5	Wolfe, 1980

1.3. PRODUCTION DATA

Data available from the U.S. EPA TSCA Production File (public portion) regarding trimethyl phosphate are as follows:

Stauffer Chem. (Gallipolis Ferry, WV)	manufacturer	10-100 thousand lbs.
Filo Chem. (New York, NY)	importer	none
Unnamed Co. (confidential)	importer	<1000 lbs.

Stauffer Chemical Co. in Gallipolis Ferry, WV, is cited as a manufacturer of alkyl phosphates (SRI, 1984); however, it is not known if trimethyl phosphate is currently manufactured at this site.

Trimethyl phosphate can be prepared by the reaction of phosphorus trichloride and an alcohol (such as methanol) to produce a disubstituted hydrogen phosphite, followed by chlorination to produce disubstituted chlorophosphate, and finally reaction with more alcohol to yield the trimethyl phosphate (Jenkins, 1947).

1.4. USE DATA

Trimethyl phosphate can be used for controlling spark plug fouling, surface ignition and rumble in gasoline engines (Hawley, 1977) when used as a gasoline additive. It also is reportedly effective as a flame retardant

(Weil, 1980); triethyl and trioctyl phosphate have been used commercially as flame retardants in polyester, cellulosic and vinyl applications (Weil, 1980).

2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

2.1. WATER

2.1.1. Hydrolysis. Ehrenberg et al. (1974) examined the hydrolysis of trimethyl phosphate in distilled water and determined the rate constants, corresponding to 20°C and 37°C, to be 3.2×10^{-5} $hour^{-1}$ and 2.4×10^{-4} $hour^{-1}$, respectively. The respective half-lives at 20°C and 37°C are ~900 and 120 days.

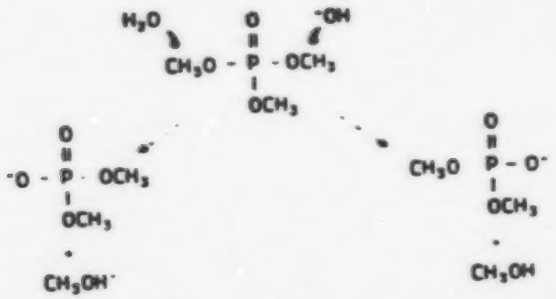
Barnard et al. (1955) experimentally determined the neutral hydrolysis rate constant for trimethyl phosphate at 100°C to be 2.3×10^{-3} min, which corresponds to a half-life of ~5 hours. Testing in acidic conditions demonstrated that the hydrolysis is not acid-catalyzed. Dimethyl phosphate was the primary hydrolysis product in neutral, acidic or basic conditions. At 35°C, the base-catalyzed hydrolysis was found to give strictly second order kinetics.

In pure water the hydrolysis proceeds by an S_N2 displacement (Lyznicki et al., 1974), which may be depicted as follows:

$$(CH_3O)_2\text{-}P(=O)\text{-}O\text{-}CH_3 + H_3O \rightarrow (CH_3)_2\text{-}PO_2^- + CH_3OH_2^+$$

Hydrolysis of trialkyl phosphates in the environmental pH range of 4-10 occurs through fission of carbon-oxygen bonds and is relatively pH independent in this range (Midwest Research Institute, 1977). At 25 and 44.7°C, the calculated hydrolysis half-lives of trimethyl phosphate in water (pH 7) have been reported to be 320 and 50 days, respectively, based on respective rate constants of $2.5 \times 10^{-8} sec^{-1}$ and $1.6 \times 10^{-7} sec^{-1}$ (Midwest Research Institute, 1977).

The neutral and alkaline hydrolysis of trimethyl phosphate can be represented as follows (Wolfe, 1980):



The second-order alkaline hydrolysis rate constant for trimethyl phosphate at 27°C is reported to be $2 \times 10^{-4} M^{-1} sec^{-1}$ (Wolfe, 1980); at pH 10, the half-life can be calculated to be ~400 days.

Mabey and Mill (1978) reported the neutral and base-catalyzed hydrolysis rate constants for trimethyl phosphate at 25°C to be $1.8 \times 10^{-6} sec^{-1}$ and $1.3 \times 10^{-4} M^{-1} sec^{-1}$, respectively. The neutral rate constant corresponds to a half-life of 1.22 years.

2.1.2. Oxidation. Pertinent data regarding the oxidation of trimethyl phosphate in the aquatic environment were not located in the available literature as cited in the Appendix.

2.1.3. Photolysis. The absorption spectrum of trimethyl phosphate in aqueous solution shows only a weak absorptivity in the region 230-300 nm (Benschop and Halmann, 1974) with apparently little absorption at >300 nm. Benschop and Halmann (1974) exposed a 0.1 M aqueous solution of trimethyl phosphate to irradiation of 254 nm for 24 hours and identified the following photolysis products: dimethyl hydrogen phosphate, methyl dihydrogen phosphate, orthophosphoric acid, CO_2, CO, formic acid, formaldehyde,

methanol and peroxides. No studies that considered irradiation at >290 nm or photosensitized photolysis were located. Therefore, the importance of aqueous photolysis is not clear. The weak absorptivity >300 nm suggests that direct photolysis is not environmentally rapid.

2.1.4. Microbial Degradation. Pertinent data regarding the microbial degradation of trimethyl phosphate in the aquatic environment were not located in the available literature as cited in the Appendix.

2.1.5. Transport.

2.1.5.1. VOLATILIZATION -- Wolfenden and Williams (1983) experimentally determined the equilibrium constant for the transfer of trimethyl phosphate to the vapor-phase from dilute aqueous solution at 20°C to be 3×10^{-7}; this value is the unitless Henry's Law Constant. Conversion to units yields a Henry's Law Constant of 7.2×10^{-9} atm-m³/mol. This value of Henry's Constant indicates that trimethyl phosphate is less volatile than water (Lyman et al., 1982); therefore, volatilization in the aquatic environment will not be significant.

2.1.5.2. ADSORPTION -- Experimental data were not located in the available literature as cited in the Appendix. The low K_{ow} and high water solubility of trimethyl phosphate suggest that adsorption by particulate organic matter and sediment in the aquatic environment is not significant.

2.1.5.3. BIOACCUMULATION -- Experimental data were not located in the available literature as cited in the Appendix. The BCF for an organic chemical can be estimated from the K_{ow} by the following regression equation derived by Veith et al. (1980): $\log BCF = 0.76 \log K_{ow} - 0.23$. For trimethyl phosphate, $\log K_{ow}$ has been reported to be -0.52 to -0.78 (Hansch and Leo, 1981); assuming $\log K_{ow}$ is -0.65, the BCF is calculated to be ~0.2, which indicates that trimethyl phosphate is not expected to bioaccumulate significantly in aquatic organisms.

2.2. AIR

Pertinent experimental data regarding the rate and transport of trimethyl phosphate in the atmosphere were not located in the available literature as cited in the Appendix. Sufficient data are not available to estimate reaction rates with hydroxyl or other radicals or with ozone. The high water solubility of trimethyl phosphate suggests that physical removal by dissolution (such as rainfall) is possible.

2.3. SOIL

Pertinent experimental data regarding the fate and transport of trimethyl phosphate in soil were not located in the available literature as cited in the Appendix. The low K_{ow} and high water solubility suggest that trimethyl phosphate may be leached readily in soil. The K_{oc} of a compound measures its propensity to bind to organic carbon present in the soil. Estimation of the K_{oc} based on the molecular topology and quantitative structure-activity relationship analysis method of Sabljic (1984) yields a K_{oc} value of ~300, which indicates a medium soil mobility (i.e., K_{oc} value between 400 and 30 and a low K_{ow} indicate moderate binding).

2.4. SUMMARY

The only environmental fate process for trimethyl phosphate that has been studied extensively under experimental conditions is aqueous hydrolysis. Under environmental conditions of the aquatic environment, the hydrolysis half-life of trimethyl phosphate is expected to be $\geq$1 year. Aqueous volatility data (Wolfenden and Williams, 1983) indicate that trimethyl phosphate will not evaporate from water to any significant extent. Pertinent data regarding the microbial degradation of trimethyl phosphate in water or soil were not located in the available literature as cited in the Appendix.

Based on its low K_{ow} and high water solubility, trimethyl phosphate is not expected to bioaccumulate in aquatic organisms and may be susceptible to leaching in soil.

3. EXPOSURE

3.1. WATER

The U.S. EPA STORET data base contains no information regarding trimethyl phosphate. Kenmotsu et al. (1982) detected trimethyl phosphate (no concentrations available) in Japanese river water and seawater. No other aquatic monitoring data were located. Exposure of trimethyl phosphate to the aquatic environment may be possible from releases from manufacturing and use operations.

3.2. FOOD

Pertinent data regarding exposure to trimethyl phosphate in food were not located in the available literature as cited in the Appendix.

3.3. INHALATION

Use of trimethyl phosphate as a gasoline additive may expose people to the compound through evaporation of the gasoline or through incompletely burned exhaust fumes (Ehrenberg et al., 1974). No monitoring data were located with respect to ambient atmospheric concentrations of trimethyl phosphate.

3.4. DERMAL

Pertinent data regarding dermal exposure to trimethyl phosphate were not located in the available literature as cited in the Appendix.

4. PHARMACOKINETICS

4.1. ABSORPTION

Jones (1970) administered ^{32}P-trimethyl phosphate by gavage to rats (100 mg/kg) and mice (1000 mg/kg). Within 16 hours, 90% of the administered dose of radioactivity was recovered in the urine of either species, indicating rapid and extensive absorption of trimethyl phosphate from the GI tract.

4.2. DISTRIBUTION

Pertinent data regarding the distribution of trimethyl phosphate were not located in the available literature as cited in the Appendix.

4.3. METABOLISM

Following oral or i.p. dosing of mice with ^{32}P-trimethyl phosphate (^{32}P-TMP), the only radioactive metabolite found in the urine and urinary bladder cannulated 1 hour after oral dosing was ^{32}P-dimethyl phosphate (Jackson and Jones, 1968; Jones, 1970). ^{32}P-Dimethyl phosphate was also the only radioactive compound found in the urine of rats treated orally with ^{32}P-trimethyl phosphate; however, when ^{32}P-trimethyl phosphate was administered intraperitoneally to the rats, both ^{32}P-trimethyl phosphate and ^{32}P-dimethyl phosphate were found in the urine within 6 hours of administration. ^{32}P-TMP is rapidly excreted as its metabolites by both rat and mouse with 90% recovery of radioactive material in urine within 16 hours and nearly complete recovery over 96 hours.

When uniformly labeled ^{14}C-trimethyl phosphate was administered to rats and mice by an unspecified route, ^{14}C-S-methyl cysteine and ^{14}C-S-methyl cysteine N-acetate were identified as urinary metabolites (Jones, 1970). Jackson and Jones (1968) reported that S-methyl cysteine appears in the mouse urine within a few hours of dosing but that production of S-methyl cysteine in the rat is slower.

In *in vitro* experiments, liver and intestinal tissue, but not kidney tissue, metabolized trimethyl phosphate (Jackson and Jones, 1968), and trimethyl phosphate reacted quantitatively with glutathione, yielding dimethyl phosphate and S-methyl glutathione, the precursor of S-methyl cysteine (Jones, 1970).

4.4. EXCRETION

As reported in Section 4.1., following oral or i.p. dosing of mice and rats with ^{32}P-trimethyl phosphate, 90% of the administered dose of radioactivity was recovered in urine collected over 16 hours (Jones, 1970). Recovery was nearly complete within 96 hours.

5. EFFECTS

5.1. CARCINOGENICITY

In an NCI (1978) bioassay, groups of 50 male and 50 female Fischer 344 rats were treated with trimethyl phosphate by gavage at dosages of 50 or 100 mg/kg/day, 3 days/week for 104 weeks and observed for an additonal week. Vehicle-control groups consisted of 20 male and 20 female rats given distilled water by gavage, 3 days/week for 105 weeks. No rats died prior to 52 weeks. Survival at the end of the study was 40% for control males, 56% for low-dose males, 35% for high-dose males, 60% for control females, 72% for low-dose females and 55% for high-dose females. All rats that died or were killed when moribund after 1 year, or were killed at the end of the study, were necropsied; comprehensive histological examination was performed. As indicated in Table 5-1, there was a statistically significant positive dose-related trend for the incidence of subcutaneous fibromas in male rats. The incidence of fibromas in high-dose males was significantly increased compared with controls. No other significantly increased incidences of tumors were observed in male or female rats.

Groups of 50 male and 50 female B6C3F1 mice were treated by gavage with trimethyl phosphate in distilled water at dosages of 250 or 500 mg/kg/day, 3 days/week for 103 weeks (NCI, 1978). No observation period followed treatment. Vehicle controls consisted of 20 male and 20 female mice treated by gavage with distilled water. Survival at the end of the study was 70% for control mice, 88% for low-dose males, 80% for high-dose males, 90% for control females, 62% for low-dose females and 59% for high-dose females. All mice that died spontaneously or were killed when moribund and all mice that were killed at the end of the study were necropsied; comprehensive histological examination was performed. No significant increased incidences of

TABLE 5-1

Oral Exposure to Trimethyl Phosphate[a]

Species/ Strain	Sex	Dose[b] (mg/kg/day)	Duration of Treatment	Duration of Study (weeks)	Purity of Compound (weeks)	Vehicle or Physical State	Target Organ	Tumor Type	Tumor Incidence[c] (P value)
rat/ Fischer 344	M	0	105	105	NA	distilled water	subcutaneous tissue	fibroma	0/20 (p=0.006)[d]
		50	104	105	96-99%				2/50 (NS)
		100	104	105	96-99%				9/49 (p=0.036)[e]
mouse/B6C3F1	F	0	103	103	NA	distilled water	uterus/ endometrium	adenocarcinoma	0/16 (p=0.003)[d]
		250	103	103	96-99%				7/40 (NS)
		500	103	103	96-99%				13/37 (p=0.004)[e]

QUALITY OF EVIDENCE

Strengths of Study: Trimethyl phosphate was administered by a relevant route at two dose levels to both sexes of two species of rodents for the lifespan of the animals. A sufficient number of animals were at risk for late developing tumors. The high dose was the MTD (determined from subchronic studies). Histological examination was comprehensive.

Overall Adequacy: Adequate

[a]Source: NCI, 1978

[b]The compound was administered 3 days/week.

[c]Expressed as number of tumor-bearing animals/number of animals with tissues examined microscopically

[d]Significant dose-related trend by Cochran-Armitage Test

[e]Significantly different from control by Fisher's Exact Test

tumors were observed in male mice; however, a statistically significant dose-related increased incidence of adenocarcinomas of the uterus/endometrium was observed in female mice (see Table 5-1). The incidence in high-dose females was significantly different from the incidence in controls. Vascular involvement and pulmonary metastases were observed in one low-dose female and four high-dose females. In addition, one squamous-cell carcinoma of the uterus/endometrium occurred in the high-dose females. One low-dose female had a leiomyosarcoma of the uterus.

5.2. MUTAGENICITY

Trimethyl phosphate has been studied extensively in mutagenicity and genotoxicity assays, the details of which are summarized in Table 5-2. Trimethyl phosphate was positive for reverse mutation in strain TA100 of Salmonella typhimurium in the presence and absence of S9 enzymes from Aroclor-induced rats (DeFlora, 1981; DeFlora et al., 1984; Purchase et al., 1978; Zeiger et al., 1982) and in the presence of liver microsomes from mice, rats or monkeys (Farrow et al., 1976). Bruce and Heddle (1979) found a positive response in TA100 in the presence, but not the absence, of S9. This positive response in TA100 suggests that TMP is a base-pair-substitution mutagen. Testing in most other strains of S. typhimurium have yielded negative results (Table 5-2), with the exception of a positive result without activation in strain C117 (Hanna and Dyer, 1975) and with activation in strains TA1535 (Purchase et al., 1978) and TA92 (Farrow et al., 1976). Farrow (1975) found that trimethyl phosphate was negative for reverse mutation in the host-mediated assay using S. typhimurium strain GS46 with the mouse as the host. Positive results for reverse mutation were obtained in Escherichia coli strains WP_2, WP_2UvrA, CM611 and WP_{12} without activation (Hanna and Dyer, 1975) and in Serratia marcesans strains Hy/α13

TABLE 5-2

Mutagenicity Testing of Trimethyl Phosphate

Assay	Indicator/ Organism	Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	Salmonella typhimurium TA1535	NR	plate incorporation	0.05, 0.5, 5, 50 and 500 µg/plate	±S9	-	S9 from Aroclor-induced rats	Bruce and Heddle, 1979
	TA1537				±S9	-		
	TA98				±S9	-		
	TA100				±S9	+		
Reverse mutation	S. typhimurium C117	NR	spot test	NR	none	+	NC	Hanna and Dyer, 1975
	646					-		
	TA1530					-		
	TA1535					-		
	TA1531					-		
	TA1532					-		
	TA1534					-		
	TA1536					-		
	TA1537					-		
	TA1538					-		
Reverse mutation	S. typhimurium TA1535	NR	plate incorporation	0.6-1.1x10^6 nmol/plate	±S9	-	S9 from Aroclor-induced rats; addition of S9 slightly enhanced the response in TA100	Deflora, 1981; Deflora et al., 1984
	TA1537				±S9	-		
	TA1538				±S9	-		
	TA98				±S9	-		
	TA100				±S9	‡		
Reverse mutation	S. typhimurium TA1535	NR	plate incorporation	4-2500 µg/plate	±S9	-	S9 from Aroclor-induced rats	Purchase et al., 1978
	TA1538				±S9	-		
	TA98				±S9	-		
	TA100				±S9	+		
Reverse mutation	S. typhimurium TA1535	NR	plate incorporation	0-250 nmol/plate	±S9	-	Addition of S9 from Arochlor-induced rats slightly enhanced the response in TA100	Zeiger et al., 1982
	TA100				±S9	‡		
Reverse mutation	S. typhimurium G546	NR	NR	125 mg/plate[a]	liver microsomes	-	microsomes for mouse, rat or monkey liver	Farrow et al., 1976
	TA92					+		
	TA100					+		
	TA98					-		

Assay	Indicator/ Organism	Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation (host-mediated)	S. typhimurium G546	NR	mouse treated i.m.	1250, 1500 or 1750 mg/kg	NA	-	NC	Farrow, 1975
Reverse mutation	Escherichia coli WP_2 WP_2uvrA CM561 CM571 CM611 WP67 WP12	NR	spot test	NR	none	+ + - - + - +	NC	Hanna and Dyer, 1975
Reverse mutation	Neurospora crassa	NR	NR	NR	none	+	NC	Kolmark, 1956
Reverse mutation	Serratia marcesans Hy/a13 Hy/a21	NR	NR	NR	NR	+	details not provided in the abstract	Bean, 1972
DNA repair	E. coli WP_2 (repair proficient) WP_{67} or CM871 (repair deficient)	NR	liquid micromethod	NR	±S9	‡	S9 from Aroclor-induced rats	Bef;ora et al., 1984
DNA repair	E. coli P3110 ($polA^+$) P3478 ($polA^-$)	NR	spot test	25 µg	none	-	NC	Fluck et al., 1976
Forward mutation	Klebsiella pneumoniae	NR	plate incorporation	2% (v/v) and 1 mol/l	none	+	NC	Voogd et al., 1972
Forward mutation	Tradescantia Clone 4430	NR	vapor phase	13 ppm, 6 hours	NA	+	p=0.02	Van't Hof and Schairer, 1982
Recessive lethal	Drosophila melanogaster	NR	feeding	NR	NA	+	NC	Lee et al., 1983
Recessive lethal	D. melanogaster	NR	feeding	≤0.1M	NA	+	NC	Hanna and Dyer, 1975; Dyer and Hanna, 1972; Dyer, 1975

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TABLE 6-2 (cont.)

Assay	Indicator/ Organism	Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Dominant lethal	ICR/Ha mice	NR	i.p. injection or gavage	200-2000 mg/kg single dose or 500 or 1000 mg/kg/day, 5 days	NA	+	dose-related increased numbers of early fetal deaths and pre-implantation losses restricted to matings during post-meiotic spermatogenesis (first 3 weeks of mating)	Epstein et al., 1970
Dominant lethal	mouse	NR	oral	500 mg/kg/day, 5 days	NA	+	significant dominant lethality in first and second weeks of mating	Farrow, 1975
Dominant lethal	mouse	NR	oral	NR, treatment for 5 days	NA	+	dominant lethal effects for 2 weeks after 5-day treatment	Newell et al., 1976
Dominant lethal	mouse, Q strain	≥99%	i.p. injection	1000 mg/kg	NA	+	significant increased frequency of pre-implantation losses 2 weeks after injection	Moutschen-Dahmen et al., 1981
Dominant lethal	mouse CF1	NR	i.p. injection	1000 mg/kg	NA	+	increased number of early fetal deaths at second week of mating	Dean and Thorpe, 1972
Sperm abnormality	B6CF1 male mice	NR	i.p. injection	0-10,000 mg/kg	NA	+	+ at ≥5000 mg/kg	Bruce and Heddle, 1979; Wyrobek and Bruce, 1975
Micronucleus test	mouse, bone marrow	NR	in vivo route NR	0, 1250, 1500 or 1750 mg/kg	NA	+	dose-related increase	Farrow et al., 1976
Micronucleus test	$B_6D_2F_1IJ$ mouse, bone marrow	NR	i.p. injection	0-10 mg/kg	NA	+	dose-related increase at 0.1-0.5 mg/kg	Weber et al., 1975
Micronucleus test	B6C3F1 female mouse, bone marrow cells	NR	i.p. injection	0-10,000 mg/kg	NA	+	NC	Bruce and Heddle, 1979
Chromosome aberrations	male Wistar rat, bone marrow cells	NR	i.p. injection	3000 mg/kg single dose or 1500 mg/kg/day x 5	NA	+	NC	Anderson and Richardson, 1981

TABLE 5-2 (cont.)

Assay	Indicator/ Organism	Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Chromosome aberrations	mouse, Q strain, bone marrow cells	≥99%	i.p. injection	NR	NA	+	NC	Moutschen-Dahmen et al., 1981
Chromosome aberrations	mouse, # strain, spermatogonial cells	≥99%	i.p. injection	NR	NA	+	NC	Moutschen-Dahmen et al., 1981
Chromosome aberrations	mouse bone marrow cells	NR	in vivo, route NR	0, 1250, 1500 or 1750 mg/kg	NA	+	NC	Farrow, 1975
Chromosome aberrations	Osborne Mendel rat, bone marrow cell	NR	i.p. or gavage	2000 mg/kg single dose or 1000 mg/ kg/day, 5 days	NA	+	NC	Legator et al., 1973
Chromosome aberrations	male CD rats, bone marrow cells	NR	i.p. injection	≤2000 mg/kg single dose or 500 mg/kg/day, 4 days	NA	+	dose-related increse	Adler et al., 1971
Chromosome abberations	Chinese hamster spermatogonia cells	NR	oral	500 mg/kg x 2 or 1000 mg/kg x 5	NA	+	NC	Machemer and Lorke, 1975
DNA breaks, single strand	rat hepatocytes	NR	cell culture	0.03, 0.3, 3.0 mM	none	- + +	NC	Sina et al., 1983

*In a review of this mutagenicity study, Connor (1979) assumed that this dose should be 125 μg/plate

NR = Not reported

NA = Not applicable

NC = No comment

and Hy/a21 (Dean, 1972). As reviewed by Connor (1979), Kolmark (1956) reported positive results for trimethyl phosphate in a reverse mutation assay with Neurospora crassa.

Trimethyl phosphate was positive with and without S9 in a DNA repair assay using E. coli strains WP_2 and WP_{67} or CM 871 (DeFlora et al., 1984) but negative without activation in a similar assay using E. coli strains P3110 and P3478 (Fluck et al., 1976). Trimethyl phosphate induced forward mutations in Klebsiella pneumoniae (Voogd et al., 1972) and in the flowering plant, Tradescantia Clone 4430 (Van't Hof and Schairer, 1982), and recessive lethal mutations in Drosophila melanogaster (Lee et al., 1983; Hanna and Dyer, 1975; Dyer and Hanna, 1972; Dyer, 1975). Trimethyl phosphate was positive in dominant lethal assays in mice, resulting in increases in early fetal deaths and pre-implantation losses within the first 3 weeks after mating (Epstein et al., 1970; Farrow, 1975; Newell et al., 1976; Moutschen-Dahmen et al., 1981; Dean and Thorpe, 1972), corresponding to the post-meiotic stages of spermatogenesis.

Trimethyl phosphate was positive in sperm abnormality (Bruce and Heddle, 1979; Wyrobek and Bruce, 1975) and micronucleus tests (Farrow et al., 1976; Weber et al., 1975; Bruce and Heddle, 1979) and caused a variety of chromosome aberrations in bone marrow cells and spermatogonial cells in mice, rats or hamsters treated in vivo (Anderson and Richardson, 1981; Moutschen-Dahman et al., 1981; Farrow, 1975; Legator et al., 1973; Adler et al., 1971; Machemer and Lorke, 1975). Trimethyl phosphate treatment of cultured rat hepatocytes resulted in dose-related increases in single strand DNA breaks (Sina et al., 1983).

5.3. TERATOGENICITY

Pertinent data regarding the teratogenicity of trimethyl phosphate were not located in the available literature as cited in the Appendix.

5.4. OTHER REPRODUCTIVE EFFECTS

As indicated in Section 5.2., trimethyl phosphate was positive for dominant lethal mutations in male mice. Epstein et al. (1970) treated male Swiss (ICR/Ha) mice with trimethyl phosphate in distilled water at daily gavage doses of 0, 500 or 1000 mg/kg/day for 5 days or at single i.p. doses of 0, 200 or 1000 mg/kg in one experiment and 0 and 500-2000 mg/kg in another. Each male was then mated with three untreated females (replaced weekly) of the same strain for 8 weeks. No consistent effect on pregnancy was observed; however, the number of pregnant females was generally reduced at 2000 mg/kg intraperitoneally or at 500 and 1000 mg/kg/day for 5 days orally. For mice treated orally, there was a significant ($p=0.01$) dose-related decrease in the number of total implants (living fetuses and early fetal deaths) and in pre-implantation losses from the matings in the first 3 weeks after treatment. The number of early fetal deaths during the second week of mating was decreased for the 500 mg/kg/day orally-dosed group. The decrease was not observed at 1000 mg/kg/day orally because of the high incidence of pre-implantation loss and the reduced number of pregnancies in this group. Similar dose-related effects were observed following i.p. dosing. Epstein et al. (1970) concluded that trimethyl phosphate produced dominant lethal mutations during postmeiotic stages of spermatogenesis (pre-implantation losses and early fetal deaths in the second week of mating) and infertility as evidenced by reduced numbers of pregnancies at the highest i.p. dose and at the oral doses. It appeared that the dominant lethal and infertility effects were cumulative and depended upon total dose regardless of route of administration.

Dominant lethal effects were also observed in the first and second week of mating following oral treatment of male mice (strain unspecified) with trimethyl phosphate at 500 mg/kg/day for 5 days (Farrow, 1975) and at unspecified oral doses for 5 days (Newell et al., 1976). Moutschen-Dahmen et al. (1981) and Dean and Thorpe (1972) observed evidence of dominant lethal mutation in the second week of mating after i.p. treatment of male mice (Q and CF_1 strains) at 1000 mg/kg trimethyl phosphate.

In an infertility study of trimethyl phosphate, groups of eight male mice and five male rats of unspecified strains were treated orally with trimethyl phosphate (Jackson and Jones, 1968; Jones and Jackson, 1969). The rats received gavage doses of 100 or 250 mg/kg/day for 5 days, while the mice received gavage (or i.p.) doses of 1000 mg/kg/day for 5 days. Controls were not used. The males were mated with females for 1-12 weeks for rats and 1-7 weeks for mice following the first dose, and the numbers of offspring were counted. For mice, total offspring resulting from matings at 1-3 weeks after oral or i.p. dosing were absent or markedly reduced in number (0-8 offspring for eight mice). For rats, total offspring resulting from matings between 2 and 5 weeks following the first dose of either 100 or 125 mg/kg were absent or markedly reduced in number (0-5 offspring for five rats). From weeks 4-7 for mice and 6-12 for rats, fertility was restored. Therefore, trimethyl phosphate administration to male rats and mice resulted in reversible functional infertility involving spermatids and spermatozoa. It was not clear whether the results represented a dominant lethal effect.

Harbison et al. (1976) also found that trimethyl phosphate produced temporary infertility in mice, rats and rabbits. Dosing was by i.p. injection or by gavage, but it was not specified which animals or in which experiments the different routes were used. Following or during treatment, the male animals were mated with females for several weeks and fecundity

(number of pregnancies/total breeding population) was determined. An unspecified number of male Swiss mice were treated with trimethyl phosphate at doses of 750 or 1500 mg/kg/day for 5 days or 1500 mg/kg/day, 5 days/week for 4 weeks. Fecundity in controls was ~75%. At 750 mg/kg/day for 5 days, fecundity was 13% during the first week of mating, but increased to ~75% during the second week. At 1500 mg/kg/day for 5 days, fecundity was 0% during the first week, but increased to ~75% during the third week. At 1500 mg/kg/day, 5 days/week for 4 weeks, 0% fecundity persisted for 2 weeks after treatment but then increased gradually to 75% at 6 weeks.

Male Sprague-Dawley rats were treated at dosages of 600 mg/kg/day for 5 days, 100 mg/kg/day 5 days/week for 4 weeks or 750 mg/kg/week during the mating schedule (12 weeks). Fecundity in controls varied between 70 and 90%. Following treatment at 600 mg/kg for 5 days, fecundity was 0-5% during mating weeks 1-4, followed by a return to 70% by the seventh week. At 100 mg/kg/day, 5 days/week for 4 weeks, fecundity was 29% during the first mating week and returned to normal after the second week. At 750 mg/kg/week during mating, fecundity was 50% at week 1, 0% at week 3 and remained between 0 and 6% through week 12.

Male New Zealand rabbits were treated at 200 or 325 mg/kg, every 5 days during mating. Fecundity in controls was 100%. At 200 and 325 mg/kg, fecundity was 50 and 10%, respectively, at 3 weeks. Continued treatment with 325 mg/kg resulted in complete infertility at week 5, which persisted throughout treatment. One week after cessation of treatment, 100% fertility was restored.

Hanna and Kerr (1981) treated male Sprague-Dawley rats with trimethyl phosphate in distilled water by gavage at 0 or 250 mg/kg/day, 6 days/week for 30 or 60 days. Sperm obtained from the epididymis of rats treated for

30 days were morphologically abnormal (detached heads or abnormalities of head, middle piece or principal piece), while sperm from controls were normal. A lack of mating ability up to 2 weeks after cessation of treatment was observed in both groups of treated males, as evidenced by lack of vaginal plugs in females mated with the males. Histologic examination of testes revealed that rats treated for 30 days had abnormal spermiogenesis and reduced numbers of mature spermatids. In rats treated for 60 days, no germ cells were observed in the seminiferous tubules. The loss of germ cells resulted in collapse of the tubules and "the appearance of the Sertoli-cell-only condition." Sertoli cell cytoplasmic processes filled the lumen of many tubules. Thus, spermatogenesis was completely lost in the rats treated for 60 days.

As indicated in Section 5.2. (see Table 5-2), sperm abnormalities were observed in B63CF1 mice treated intraperitoneally with trimethyl phosphate at ≥5000 mg/kg (Bruce and Heddle, 1979); chromosome aberrations were observed in spermatogonial cells of strain Q mice treated intraperitoneally with an unsecified dose (Moutschen-Dahmen et al., 1981) and in Chinese hamsters treated orally with 500 mg/kg x 2 or 1000 mg/kg x 5 (Machemer and Lorke, 1975).

5.5. CHRONIC AND SUBCHRONIC TOXICITY

In the NCI (1978) carcinogenicity bioassay (see Section 5.1.), groups of 50 male and 50 female Fischer 344 rats and B6C3F1 mice were treated by gavage with trimethyl phosphate for ~2 years. Twenty animals of each sex and species served as controls. Dosages in rats were 50 or 100 mg/kg/day, 3 days/week; dosages in mice were 250 or 500 mg/kg/day, 3 days/week.

For rats, a dose-related slightly decreased mean body weight gain was observed for treated males and females. No significant differences in mortality occurred among groups. Gross and histologic examination of rats

that died during the study or were killed at the end of the study revealed no treatment-related nonneoplastic changes. Increased incidences of subcutaneous fibromas were observed in treated males (see Section 5.1.).

For mice, dose-related decreased mean body weight was observed in females but not in males. Mortality was comparable among groups. Histologic examination revealed two nonneoplastic changes that may have been secondary to the development of uterine carcinomas (see Section 5.1.) in female mice. Hydronephrosis occurred in six females, five of which had uterine tumors. Three high-dose females with pulmonary metastases of the uterine tumors had extensive thrombosis of the pulmonary artery. No other treatment-related nonneoplastic lesions were observed in mice.

In order to establish the MTD values for use in the chronic study, a subchronic study was conducted (NCI, 1978). Groups of five male and five female rats and mice were treated by gavage with trimethyl phosphate in water, 3 days/week for 7 weeks. Rats were given dosages of 0, 100, 147, 215, 316, 464, 681, 1000 or 1470 mg/kg/day. Mice were treated at 0, 147, 215, 316, 464, 681, 1000, 1470 or 2150 mg/kg/day.

For rats, dosages $\geq$681 mg/kg/day resulted in 100% mortality, while one male treated with 464 mg/kg/day died. Male and female rats at $\leq$316 mg/kg/day gained ~20% less weight than did controls. At 464 mg/kg/day, males gained ~44% and females gained ~32% less weight than did controls. Distended bladders and GI hemorrhage were observed in rats that died before the end of the study.

For mice, five males and one female at 2150 mg/kg/day and two females at 1470 mg/kg/day died. Body weight gain was slightly decreased for males at $\geq$681 mg/kg/day. Although all surviving rats and mice were killed and necropsied 1 week after cessation of treatment, the results were not reported.

Oishi et al. (1982) maintained groups of six male JCL-Wistar rats on diets containing trimethyl phosphate at concentrations of 0 or 0.5% (5000 ppm) for 9 weeks. Treated rats had significantly ($p<0.05$) decreased mean body weight and increased absolute and relative kidney weight. The absolute weight of the testes was significantly decreased compared with controls. Significant decreases in erythrocyte count, hemoglobin concentration, prothrombin time, SGOT and SGPT were observed. The toxicologic significance of decreased levels of SGOT and SGPT is unknown. Slight changes in the liver, described as cytoplasmic vacuolization, increased numbers of binucleate cells and enlarged cells, occurred in treated rats.

In the study by Harbison et al. (1976) (see Section 5.4.), male Swiss mice were treated with trimethyl phosphate at 1500 mg/kg/day, 5 days/week for 4 weeks; male Sprague-Dawley rats were treated at 100 mg/kg/day, 5 days/week for 4 weeks or 750 mg/kg/week for 12 weeks; and male New Zealand rabbits received 200 or 325 mg/kg/day, every 5 days for 9 and 13 weeks, respectively. Appropriate controls were used. It was not clear whether dosing was by gavage or i.p. injection. Following treatment for 4 weeks or during the treatments for $\geq$9 weeks, the animals were mated with untreated females and fecundity was measured. In all cases, reversible infertility was observed.

In the study by Hanna and Kerr (1981) (see Section 5.4.), male Sprague-Dawley rats were treated by gavage with trimethyl phosphate at 0 or 250 mg/kg/day, 6 days/week for 30 or 60 days. Epididymal sperm were morphologically abnormal, mating ability was impaired and spermatogenesis was impaired (30 days) or completely lacking (60 days) as evidenced by histologic examination of testes.

The neurotoxicity of trimethyl phosphate, evaluated by behavior, neurologic examination, neurophysiologic tests and neuropathology, was studied in beagle dogs by Schaeppi et al. (1984). Two males and three females were treated orally with 1 mL of trimethyl phosphate in a gelatine capsule daily for 29, 50 (males), 71, 101 or 121 (females) days. An additional female dog received 2 mL trimethyl phosphate 5 days/week for 150 days (total dose 180 mL). On the day after the last treatment, the dogs were killed and necropsied. All dogs treated daily with 1 mL developed signs of neurotoxicity, which became progressively more severe with duration of treatment. These signs were impairments of gait, hopping, tactile placing and landing, persistence in abnormal posture and decreased muscle tone. The dogs that received $\geq$50 treatments had prolonged distal latency for neuromuscular impulse transmission. Sensory MNCV was decreased for the dog receiving 120 treatments. No changes in peripheral motor MNCV occurred in any dogs receiving 1 mL/day. No neuropathologic changes were observed in dogs treated for $\leq$71 days. Dogs treated for 101 and 121 days had degenerative changes in nerve fibers and demyelination of axons.

The dog treated with 2 mL daily, 5 days/week had body weight loss after 85 days. Inactivity progressing to severe morbidity was observed after 88 days. Because of the severity of neurotoxic signs, the dosing schedule was interrupted at times during the experiment. The dog received a total of 180 mL in 150 days. Signs of neurotoxicity included enhanced patellar reflex (day 18), attenuated extensor postural thrust (day$_{\pm}$ 25), atactic gait (day 39), decreased muscle force and persistence of abnormal posture (day 46), decreased muscle tonus and impaired hopping and landing (day 53). These signs became progressively more severe as treatment continued. Sensory and central motor MNCV were decreased below pretreatment

measurements. Upon histologic examination, severe distal degeneration of spinal and peripheral nerve fibers, demyelination of nerve fibers and axonless Schwann cells were observed.

Deichmann and Witherup (1946) treated six rabbits with 20 dermal applications of 2 mL/kg trimethyl phosphate, 2 hours/day over a 28-day period. Body weight loss was observed in three rabbits, and one rabbit developed flaccid paralysis and abnormal posture. An additional group of three rabbits was treated dermally with 2 mL/kg for 3 hours/day. Severe body weight loss, tremors, unsteadiness, paralysis and death occurred within 14 treatments.

A cat treated subcutaneously with 79 injections of 0.1 mL/kg trimethyl phosphate over 123 days lost weight and developed general weakness (Deichmann and Witherup, 1946).

5.6. OTHER RELEVANT INFORMATION

LD_{50} values for trimethyl phosphate are summarized in Table 5-3. Signs of toxicity at lethal doses include decreased respiratory movement, weakness, hyperirritability, fine tremors, dyspnea, collapse and respiratory failure (Deichmann and Witherup, 1946). Administration of 0.3 mL/kg/day (363 mg/kg/day, using density of 1.21 g/mL) (see Table 5-3) for 6 days to rabbits resulted in weight loss and signs of toxicity including tremors, unsteadiness, weakness, flaccid paralysis and spasticity (Deichmann and Witherup, 1946). Verschoyle and Cabral (1982) reported that rats treated orally with a lethal dose of trimethyl phosphate did not develop signs of cholinergic poisoning. Hollingshaus and Fukuto (1982) reported that trimethyl phosphate did not cause delayed neurotoxicity in hens.

TABLE 5-3

LD_{50} Values for Trimethyl Phosphate

Species	Route	LD_{50}	Reference
Rat	oral	1.65 mL/kg (1997 mg/kg)[a]	Deichmann and Witherup, 1946
Rat	oral	2.83 mL/kg (3424 mg/kg)[a]	Smyth et al., 1969
Rat	oral	1109 mg/kg (850-1447)[b]	Verschoyle and Cabral, 1982
Rat	oral	>300 mg/kg[c]	Hollingshaus and Fukuto, 1982
Rabbit	oral	1.05 mL/kg (1271 mg/kg)[a]	Deichmann and Witherup, 1946
Rabbit	dermal	2.83 mL/kg (3424 mg/kg)[a]	Smyth et al., 1969

[a]Dose in mg/kg calculated by using the density of trimethyl phosphate at 68°F of 1.21 g/mL (see Section 1.2.).

[b]95% confidence limits

[c]Highest dose tested

6. AQUATIC TOXICITY

6.1. ACUTE TOXICITY

Few data regarding the toxic responses of aquatic organisms to trimethyl phosphate were located in the available literature. Hemsworth and Wardhaugh (1978) investigated the effect of the antifertility agent on spermatozoa from the gametes of Xenopus laevis as manifested in developing embryos. In an in vitro system, spermatozoa isolated from mascerated testes were exposed to trimethyl phosphate in a suspension maintained at 25±2°C for 30 minutes. Nominal concentrations of 0 (control), 10, 50, 75 and 120 mg/mL were tested. Observations of embryonic development, using a dissection microscope, were recorded throughout periods of cleavage, gastrulation and neurulation to tadpole formation; the overall sequence required 48 hours to complete. No developmental abnormalities were observed throughout the range of concentrations tested. Although the highest concentration of trimethyl phosphate used (120 mg/mL) interfered with motility, successful fertilization and normal embryonic development did not differ significantly from the controls.

6.2. CHRONIC TOXICITY

Hanna (1982) reported a disruption of spermatogenesis in adult male guppies, Poecilia reticulata, exposed to trimethyl phosphate. Exposure to a nominal concentration of 2000 ppm and a control were maintained at 25±2°C throughout the 100-day test period. Test concentrations were renewed weekly. Fish of both the control and treatment groups were sacrificed on day 100. Observations of testicular development and size and body length were recorded. Permanent mounts of stained sections of the testes, embedded with a low viscosity medium, were prepared.

The testes exhibited a significant decrease in length compared with controls, which indicated a loss in testicular volume. There was no significant difference in body length, however. Upon microscopic examination, the fish treated with trimethyl phosphate exhibited gross pathological changes, including a marked reduction in cyst numbers that accounted for the reduction in testicular size. No normal meiotic or postmeiotic cysts were observed, but several degenerating cysts were noted. Furthermore, a high degree of vacuolization was observed in the degenerating cyst cells and the epithelial cells of the efferent ducts.

No deaths occured during the treatment of male fish with trimethyl phosphate; however, movement became lethargic late in the exposure at 120 mg/mℓ (Hanna, 1982).

6.3. PLANT EFFECTS

Data regarding the toxicity of trimethyl phosphate to aquatic plants were not located in the available literature as cited in the Appendix.

6.4. RESIDUE

Data regarding trimethyl phosphate residues in aquatic organisms were not located in the available literature as cited in the Appendix.

6.5. OTHER RELEVANT INFORMATION

Additional information regarding the toxic effects of trimethyl phosphate to aquatic organisms were not located in the available literature as cited in the Appendix.

7. EXISTING GUIDELINES AND STANDARDS

7.1. HUMAN

Pertinent guidelines and standards, including U.S. EPA ambient water and air quality criteria, drinking water standards, FAO/WHO ADIs, EPA or FDA tolerances for raw agricultural commodities or foods, and ACGIH, NIOSH or OSHA occupational exposure limits were not located in the available literature as cited in the Appendix.

7.2. AQUATIC

Guidelines and standards for the protection of aquatic organisms from the toxic effects of trimethyl phosphate were not located in the available literature as cited in the Appendix.

8. RISK ASSESSMENT

In a bioassay for carcinogenicity (NCI, 1978), administration of trimethyl phosphate in distilled water at gavage doses of 50 and 100 mg/kg/day to rats and 250 and 500 mg/kg/day to mice, 3 days/week for ~2 years resulted in statistically significant dose-related increased incidences of subcutaneous fibromas in male rats and adenocarcinomas of the uterus/endometrium in female mice compared with vehicle-treated controls (see Table 5-1). The incidences in the high-dose male rats and female mice were significantly different from control. Vascular involvement and pulmonary metastases occurred in several female mice with tumors of the uterus/endometrium.

As summarized in Table 5-2, trimethyl phosphate was positive for reverse mutations in at least one strain (TA100) of Salmonella typhimurium, in several strains of Escherichia coli, in Neurospora crassa and in Serratia marcesans; for forward mutations, in Klebsiella pneumoniae and Tradescantia. A positive result was obtained for DNA repair in E. coli using strains WP_2 (repair proficient) and WP_{67}ovCM871 (repair deficient) but not using E. coli strains P3110 (pol A^+) and P3478 (pol A^-). Metabolic activation was not necessary for positive results in the bacterial systems. Trimethyl phosphate exposure also resulted in recessive lethal mutations in Drosophila melanogaster, dominant lethal mutations in male mice and chromosome aberrations in mice, rats and hamsters.

No pertinent data regarding the teratogenicity of trimethyl phosphate were available; however, trimethyl phosphate administration to male rats, mice and rabbits has resulted in reversible infertility at single i.p. doses $\geq$200 mg/kg or gavage doses $\geq$100 mg/kg/day for $\geq$5 days (Harbison et al., 1976; Jackson and Jones, 1968; Epstein et al., 1970). Lower doses were not tested, but Epstein et al. (1970) found the reproductive effects to be

cumulative and dependent upon total dose regardless of route of administration. Hanna and Kerr (1981) reported that male rats treated by gavage with 250 mg/kg/day, 6 days/week for 30 or 60 days had reversible loss of mating ability, morphologically abnormal sperm and impaired or complete loss of spermatogenesis.

The NCI (1978) carcinogenicity bioassay was the only available chronic study. The only nonneoplastic effect in rats was a slightly decreased body weight gain. Body weight gain was also decreased in female, but not male mice. In addition, the female mice with tumors of the uterus/endometrium also had hydronephrosis and pulmonary artery thrombosis. Decreased body weight gain was also observed in the 7-week subchronic study in rats treated at ≥100 mg/kg/day and mice treated at ≥681 mg/kg/day, 3 days/week (NCI, 1978). Higher doses (≥464 mg/kg/day for rats, ≥1470 mg/kg/day for mice) were fatal. Dietary administration of trimethyl phosphate at 5000 ppm for 9 weeks to rats resulted in decreased body weight gain, increased kidney weight, decreased testes weight and decreased erythrocyte count, hemoglobin concentration, SGPT and SGOT (Oishi et al., 1982). Treatment of beagle dogs with 1 or 2 mL/day trimethyl phosphate for up to 150 days (≥93 mg/kg/day, using the density of trimethyl phosphate at 68°F of 1.21 g/mL as given in Section 1.2.) resulted in behavioral, neurologic, electrophysiologic and neuropathologic evidence of neurotoxicity, which became progressively more severe with duration of treatment (Schaeppi et al., 1984).

Because the dominant lethal and reproductive effects observed at otherwise nontoxic doses in short-term studies appeared to be cumulative and dependent upon total dose (Epstein et al., 1970), and since infertility has been shown to persist throughout the duration of exposure to trimethyl phosphate in rats and rabbits (Harbison et al., 1976), it is not possible to

define a LOAEL or NOAEL for chronic exposure to trimethyl phosphate. Since trimethyl phosphate was shown to be carcinogenic to female mice (NCI, 1978), however, it is appropriate to derive a q_1^*.

A q_1^* for humans of $3.73 \times 10^{-2}(mg/kg/day)^{-1}$ was derived from the dose-response data for adenocarcinoma of the uterus/endometrium in female mice (Table 8-1). The dosages of 250 and 500 mg/kg/day, 3 days/week were transformed to TWA dosages of 107 and 214 mg/kg/day, respectively, by expanding the exposure over 7 days. The unadjusted q_1^* was calculated using the linearized multistage model developed by Kenneth Crump and adopted by the U.S. EPA (Federal Register, 1980). The q_1^* value for humans was calculated by multiplying the unadjusted value by the cube root of the ratio of the human weight (assumed to be 70 kg) to the average weight of the female mice in the study (0.03 kg) to correct for interspecies differences, and by the cube of the ratio of the lifespan of the mouse (assumed to be 104 weeks) to the duration of the study (103 weeks).

TABLE 8-1

Cancer Data Sheet for Derivation of q_1^*

Compound: trimethyl phosphate

Reference: NCI 1978

Species, Strain, Sex: mouse, B6C3F1, Female

Body weight: 0.03 kg (measured)

Length of exposure (le) = 103 weeks

Length of experiment (Le) = 103 weeks

Lifespan of animal (L) = 104 weeks

Tumor site and type: uterus/endometrium, adenocarcinoma

Route, vehicle: oral (gavage), distilled water

Experimental Doses or Exposures (mg/kg/day, 3 days/week)	Transformed Dose (mg/kg/day)	Incidence: No. Responding/No. Tested or Examined
0	0	0/16
250	107	7/40
500	214	13/37

Unadjusted q_1^* from study = 2.7350876×10^{-3} $(mg/kg/day)^{-1}$

Human q_1^* = 3.73438×10^{-2} $(mg/kg/day)^{-1}$

9. REPORTABLE QUANTITIES

9.1. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY

The available chronic, subchronic and reproductive studies of trimethyl phosphate were discussed in Chapter 5 and are summarized in Table 9-1. Although the RQ is based on chronic toxicity, several short-term studies are considered candidate studies for RQ ranking because the effects on male fertility appear to be cumulative and dependent upon total dose. Harbison et al. (1976) found that oral administration of trimethyl phosphate to male rats, mice or rabbits at various dosages for up to 13 weeks resulted in functional reversible infertility. Hanna and Kerr (1981), however, found that treatment of rats with 250 mg/kg/day, 6 days/week for 30 days resulted in microscopically reduced spermatogenesis, while treatment for 60 days resulted in complete loss of spermatogenic elements. Chronic treatment of rats with 50 or 100 mg/kg/day, 3 days/week for 2 years did not result in histopathology of the testes. However, this study was not designed to thoroughly evaluate this endpoint (NCI, 1978). The rats and mice in the NCI (1978) study were not evaluated for functional fertility. Although the studies of Harbison et al. (1976) are of short duration (4-12 weeks), since they examined the critical effect and since they employed doses lower than those in the longer duration studies, they will be included as candidates for CS calculation. The other study that examined fertility is deemed less appropriate because of only 5 days of dosing (Jackson and Jones, 1968).

The lowest dose tested in mice that resulted in reversible decreased fertility (500 mg/kg/day for 5 days) also induced dominant lethal mutations (Epstein et al., 1970). This endpoint reflects the potential mutagenicity of the compound and is not appropriately included as a benchmark for chronic toxicity. In addition, the doses used were higher than the fertility study and, therefore, would not result in a more protective CS.

TABLE 9-1

Toxicity Summary for Trimethyl Phosphate

Route	Species/ Strain	Sex	No. at Start	Vehicle Physical State	Purity	Dosage/Exposure	Transformed Dose mg/kg/day[a]	Response	Reference
Oral (gavage)	rat/ Fischer 344	M/F	50 M, 50 F per group	distilled water	~96-99%	50 or 100 mg/kg/day, 3 days/week, 104 weeks	21.4 or 42.9	slightly decreased body weight gain, females	NCI, 1978
			5 M, 5 F per group			≥100 mg/kg/day, 3 days/week, 7 weeks	≥42.9	decreased body weight gain	NCI, 1978
			5 M, 5 F			464 mg/kg/day, 3 days/week, 7 weeks	198.9	death in 1/5 males, distended bladder, GI-hemorrhage, severely decreased body weight	NCI, 1978
			5 M, 5 F per group			≥681 mg/kg/day, 3 days/week, 7 weeks	≥291.9	100% mortality, distended bladder, GI hemorrhage	NCI, 1978
Oral or i.p.[b]	rat/ Sprague-Dawley	M	NR	NR	97%	600 mg/kg/day for 5 days, 100 mg/kg/day for 5 days/week for 4 weeks or 750 mg/kg/week for 12 weeks	600, 71 or 107	reversible infertility	Harbison et al., 1976
	rat/ Sprague-Dawley	M	NR	distilled water	NR	250 mg/kg/day, 6 days/week, 30 or 60 days	214.3	reversible loss of mating ability, morphologically abnormal sperm, impaired or loss of spermatogenesis	Hanna and Kerr, 1981

TABLE 9-1 (cont.)

Route	Species/ Strain	Sex	No. at Start	Vehicle Physical State	Purity	Dosage/Exposure	Transformed Dose mg/kg/day[a]	Response	Reference
Oral (gavage)	mouse/ B6C3F1	M/F	50 M, 50 F per group	distilled water	~96-99%	250 or 500 mg/kg/day, 3 days/week, 103 weeks	107.1 or 214.3	decreased body weight gain (females), hydronephrosis and pulmonary artery thrombosis secondary to tumor development in females	NCI, 1978
			5 M, 5 F per group			≥681 mg/kg/day, 3 days/week, 7 weeks	≥291.9	slightly decreased body weight gain (males)	NCI, 1978
			5 M, 5 F			1470 mg/kg/day, 3 days/week, 7 weeks	630	death in 2/5 females	NCI, 1978
			5 M, 5 F			2150 mg/kg/day, 3 days/week, 7 weeks	921.4	death in 5/5 males, 2/5 females	NCI, 1978
Oral or i.p.[a]	mouse/ Swiss	M	NR	NR	97%	750 or 1500 mg/kg/day for 5 days or 1500 mg/kg/day, 5 days/week for 4 weeks	750, 1500 or 1071.4	impaired reproductive capacity at 750 mg/kg/day, infertility at 1071.4 and 1500 mg/kg/day	Harbison et al., 1976
Oral (gavage)	mouse/ Swiss ICR/ Ha	M	NR	distilled water	NR	500 or 1000 mg/kg/day, 5 days	500 or 1000	reduced number of pregnancies in females mated to treated rats, dominant lethal mutation (preimplantation loss and early fetal death)	Epstein et al., 1970
Oral or i.p.[a]	rabbit/New Zealand	M	NR	NR	97%	200 or 325 mg/kg, every 5 days for 9 and 13 weeks, respectively	~41.3 or 68.6[b]	reversible infertility	Harbison et al., 1976

TABLE 9-1 (cont.)

Route	Species/ Strain	Sex	No. at Start	Vehicle Physical State	Purity	Dosage/Exposure	Transformed Dose mg/kg/day[a]	Response	Reference
Oral	dog/beagle	M/F	5	gelatine capsule	NR	1 ml/day, for 29, 50, 71, 101, or 121 days	~93.1[c]	behavioral, neurologic, electrophysiolic and neuropathologic evidence of neurotoxicity	Schaeppi et al., 1984
		F	1			2 ml/day, 5 days/week, (180 ml in 150 days)[d]	~152.8[c]	greater severity of neurotoxicity	Schaeppi et al., 1984

[a]Dosing was by gavage or i.p. injection, but it was not specified which animals or in which experiments the different routes were used. Similar effects, however, are observed following oral or i.p. administration (see Sections 5.2. and 5.4.).

[b]The doses were calculated assuming that the rabbits received 13 doses of 200 mg/kg in 63 days or 19 doses of 325 mg/kg in 90 days.

[c]The doses were calculated using the density of trimethyl phosphate at 68°F (1.21 g/ml) (see Section 1.2.) and the average weight of the dogs in the study (13 kg for dogs treated with 1 ml/day, 9.5 for the dog given a total of 180 ml).

[d]Because of the severity of effects, dosing schedule was interrupted at times during the experiment.

NR - Not reported

M - Male

F - Female

Another effect of trimethyl phosphate treatment was decreased body weight gain, which was observed in rats at transformed doses of 21.4 or 42.9 mg/kg/day for 2 years or at ≥42.9 mg/kg/day for 7 weeks, and in mice at transformed doses of 107.1 or 214.3 mg/kg/day for 2 years or at ≥291.9 mg/kg/day for 7 weeks. The 2-year low dose in rats can be used to calculate an MED for reduced body weight, but the 2-year low dose in mice cannot be used because hydronephrosis and pulmonary artery thrombosis associated with uterine carcinomas and pulmonary metastases occurred at this dose. The dose of 291.9 mg/kg/day for 7 weeks in mice, however, can be used. The deaths observed at higher doses in mice and rats in the 7-week study represent an acute response to relatively high doses and, therefore, cannot be used for RQ derivation.

Schaeppi et al. (1984) observed behavioral, electrophysiologic and microscopic neuropathologic evidence of neurotoxicity in dogs treated orally with trimethyl phosphate at ≥93.1 mg/kg/day. The dose is adjusted by a factor of 10 to account for the subchronic exposure duration.

The data used to calculate CSs are given in Table 9-2. The MEDs were calculated from the animal transformed doses by multiplying by the cube root of the ratio of the animal weight to the average weight of a man (assumed to be 70 kg). The weights used for the animals were 0.25 kg for female rats in the NCI (1978) study, 0.35 kg for male rats (assumed), 0.03 kg for mice (assumed), 3.7 kg for rabbits (assumed) and 13 kg for the dogs, based on data from Schaeppi et al. (1984). The resultant human dose in mg/kg/day was converted to the MED in units of mg/day by multiplying by 70 kg. When appropriate, the doses were divided by an uncertainty factor of 10. The RV_ds corresponding to the MEDs are given in Table 9-2. The effect on fertility of male rats, mice and rabbits and the neurotoxicity in dogs

TABLE 9-2

Composite Scores for the Oral Toxicity of Trimethyl Phosphate

Species	Dose/Exposure (mg/kg/day)	Human MED (mg/day)	RV_d	Effect	RV_e	CS	RQ	Reference
rat	21.4/104 weeks	229.0	2.0	decreased body weight gain	4	8	1000	HCI, 1978
	71/4 weeks	85.0*	2.6	reversible infertility	9	23.4	100	Harbison et al., 1976
	214.3/60 days	256*	1.9	loss of spermatogenesis	9	17	1000	Hanna and Kerr, 1981
mouse	500/5 days	263.9*	1.9	pre-implantation losses, early fetal death	9	17.1	1000	Epstein et al., 1970
	291.9/7 weeks	154.1*	2.2	decreased body weight gain	4	8.8	1000	HCI, 1978
rabbit	41.3/9 weeks	108.5*	2.4	reversible infertility	9	21.6	100	Harbison et al., 1976
dog	93.1/≤120 days	371.8*	1.6	neuropathy	9	14.4	1000	Schaeppi et al., 1984

*The dose was divided by an uncertainty factor of 10 to approximate chronic exposure.

warrant an RV_e of 9 in each case. Effects on body weight warrant an RV_e of 4. The CSs were calculated by multiplying the RV_d by the RV_e. The highest CS of 23.4 resulted from the data for reversible infertility in male rats in the study by Harbison et al. (1976), corresponding to an RQ of 100 (Table 9-3).

9.2. WEIGHT OF EVIDENCE AND POTENCY FACTOR ($F=1/ED_{10}$) FOR CARCINOGENICITY

The available data regarding the carcinogenicity of trimethyl phosphate were discussed in Section 5.1. As indicated in Table 5-1, administration of trimethyl phosphate by gavage to male and female mice at 0, 250 and 500 mg/kg/day, 3 days/week for 103 weeks resulted in induction of adenocarcinomas of the uterus/endometrium in females (NCI, 1978). Vascular involvement and pulmonary metastases occurred in several of the mice that had uterine tumors. In addition, treatment of male and female rats with trimethyl phosphate by gavage at 0, 50 and 100 mg/kg/day, 3 days/week for 104 weeks resulted in increased incidences of subcutaneous fibromas in male rats (see Table 5-1). Therefore, there is sufficient evidence that trimethyl phosphate is carcinogenic to animals. No data were available regarding the carcinogenicity of trimethyl phosphate to humans. Therefore, trimethyl phosphate is classified as an IARC Group 2B chemical. Under the current U.S. EPA guidelines for classifying carcinogens (Federal Register, 1984), trimethyl phosphate falls into the B2 category meaning that it is a "probable" human carcinogen.

As indicated in Table 9-4, the F factor for trimethyl phosphate of 2.2×10^{-1} $(mg/kg/day)^{-1}$ was calculated from the dose-response data for adenocarcinomas of the uterus/endometrium in female mice (NCI, 1978). The unadjusted $1/ED_{10}$ was calculated using the linearized multistage model

TABLE 9-3

Trimethyl Phosphate

Minimum Effective Dose (MED) and Reportable Quantity (RQ)

Route:	oral
Dose*:	85.0
Effect:	infertility
Reference:	Harbison et al., 1976
RV_d:	2.6
RV_e:	9
Composite Score:	23.4
RQ:	100

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*Equivalent Human Dose

TABLE 9-4

Derivation of Potency Factor (F)
Agent: Trimethyl Phosphate

Reference:	NCI, 1978		
Exposure route:	oral (gavage)		
Species:	mouse		
Strain:	B6C3F1		
Sex:	female		
Vehicle or physical state:	distilled water		
Body weight:	0.03 kg (measured)		
Duration of treatment:	103 weeks		
Duration of study:	103 weeks		
Lifespan of animal:	104 weeks		
Target organ:	uterus/endometrium		
Tumor type:	adenocarcinoma		
Experimental doses/ exposure:	0,	250,	500 mg/kg/day, 3 days/week
Transformed doses:	0	107	214 mg/kg/day
Tumor incidence:	0/16	7/40	13/37
Unadjusted $1/ED_{10}$:	1.6163748×10^{-2}		
F factor:	2.206934×10^{-1}		

developed by Kenneth Crump and adopted by the U.S. EPA (Federal Register, 1980). The F factor was calculated by multiplying the unadjusted $1/ED_{10}$ by the cube root of the ratio of the human weight (assumed to be 70 kg) to the average weight of female mice in the study (0.03 kg) to extrapolate from mice to humans and by the cube of the ratio of the lifespan of the mouse (assumed to be 104 weeks) to the duration of the study (103 weeks).

Because the F factor of 2.2×10^{-1} $(mg/kg/day)^{-1}$ is <1, trimethyl phosphate is placed in potency group 4. A potency group 4 and an IARC group 2B chemical has a LOW hazard ranking under CERCLA.

10. REFERENCES

Adler, I.D., G. Ramarao and S. Epstein. 1971. In vivo cytogenetic effects of trimethylphosphate and of Tris(2-methyl-1-aziridinyl)phosphine oxide [TEPA] on bone marrow cells of male rats. Mutat. Res. 13(3): 263-273.

Anderson, D. and C.R. Richardson. 1981. Issues relevant to the assessment of chemically induced chromosome damage in vivo and their relationship to chemical mutagenesis. Mutat. Res. 90(3): 261-272.

Barnard, P.W.C., C.A. Bunton, D.R. Llewellyn, K.G. Oldham, B.L. Silver and C.A. Vernon. 1955. The hydrolysis of organic phosphates. Chem. Ind. p. 760.

Benschop, H.P. and M. Halmann. 1974. Photochemistry of phosphorus compounds. IX. Photolysis of trimethyl phosphate in aqueous solution. J. Chem. Soc., Perkin Trans. 2(10): 1175-1180.

Bruce, W.R. and J.A. Heddle. 1979. The mutagenic activity of 61 agents as determined by the micronucleus, Salmonella, and sperm abnormality assays. Can. J. Genet. Cytol. 21(3): 319-333.

Connor, T.H. 1979. The mutagenicity of trimethyl phosphate. Mutat. Res. 65(2): 121-131.

Dean, B.J. 1972. Mutagenic effects of organophosphorus pesticides on microorganisms. Arch. Toxicol. 30(1): 67-74.

Dean, B.J. and E. Thorpe. 1972. Studies with dichlorvos vapour in dominant lethal mutation tests in mice. Arch. Toxicol. 30: 51-59.

DeFlora, S. 1981. Study of 106 organic and inorganic compounds in the Salmonella/microsome test. Carcinogenesis. 2(4): 283-298.

DeFlora, S., P. Zanacchi, A. Camoirano, C. Bennicelli and G.S. Badolati. 1984. Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutat. Res. 133(3): 161-198.

Deichmann, W.B. and S. Witherup. 1946. Observations on the effects of trimethyl phosphate upon experimental animals. J. Pharm. Exp. Ther. 88: 338.

Dyer, K.F. 1975. Mutagenic activity of trimethylphosphate in Drosophila at low doses. Mutat. Res. 28(3): 465-468.

Dyer, K.F. and P.J. Hanna. 1972. Mutagenic and antifertility activity of trimethylphosphate in Drosphila melarogaster. Mutat. Res. 1(3): 327-31.

Ehrenberg, L., S. Osterman-golkar, D. Singh and U. Lundqvist. 1974. On the reaction kinetics and mutagenic activity of methylating and beta-halogenoethylating gasoline additives. Radiat. Bot. 15: 185-194.

Epstein, S.S., W. Bass, E. Arnold and Y. Biship. 1970. Mutagenicity of trimethylphosphate in mice. Science. 168(3931): 584-586.

Farrow, M.G. 1975. The use of trimethylphosphate (TMP) as a positive control agent in the dominant lethal, cytogenetic and host-mediated assays. Mutat. Res. 31: 314-315.

Farrow, M.G., J. Barnett, L.J. Lassen and L. Wetzel. 1976. The use of trimethylphosphate (TMP) as a positive agent, Salmonella/microsome test, the Salmonella/urine assay and the micronucleus test. Mutat. Res. 38: 381-382.

Federal Register. 1980. Guidelines and Methodology for the Preparation of Health Effect Assessment Chapters of the Ambient Water Quality Criteria Documents. U.S. EPA. 45 FR 77318.

Fluck, E.R., L.A. Poirier and H.W. Ruelins. 1976. Evaluation of a DNA polymerase-deficient mutant of E. coli for the rapid evaluation of carcinogens. Chem.-Biol. Interact. 15: 219-231.

Hanna, P.J. 1982. Effects on spermatogenesis in the guppy, Poecilia reticulata, by prolonged exposure to trimethyl phosphate. Bull. Environ. Contam. Toxicol. 28(1): 28-32.

Hanna, P.J. and K.F. Dyer. 1975. Mutagenicity of organophosphorus compounds in bacteria and Drosophila. Mutat. Res. 28(3): 405-420.

Hanna, P.J. and J.B. Kerr. 1981. Antifertility activity of trimethylphosphate in male rats. Experientia. 37: 999-1001.

Hansch, C. and A.J. Leo. 1981. Medchem Project. Issue No. 19. Pomona College, Claremont, CA.

Harbison, R.D., C. Dwivedi and M.A. Evans. 1976. A proposed mechanism for trimethyl phosphate-induced sterility. Toxicol. Appl. Pharmacol. 35: 481.

Hawley, G.G. 1977. The Condensed Chemical Dictionary, 9th ed. Van Nostrand Reinhold Co., New York. p. 889.

Hemsworth, B.N and A.A. Wardhaugh. 1978. Embryopathies due to spermatozoal impairment in Xenopus laevis. Mutat. Res. 51(1): 45-53.

Hollingshaus, J.G. and T.R. Fukuto. 1982. Effect of chronic exposure to pesticides on delayed neuro toxicity. In: Effects of Chronic Exposures to Pesticides on Animal Systems, J.E. Chambers and J.D. Yarbrough, Ed. Raven Press, New York, NY. p. 85-120.

Jackson, H. and A.R. Jones. 1968. Antifertility action and metabolism of trimethyl phosphate in rodents. Nature. 220(5167): 591-592.

Jenkins, R.L. 1947. Neutral phosphoric acid esters (trisubstituted phosphates). U.S. Patent 2,426,691. Sept. 2, 1947 (to Monsanto Chemical Co.). (CA 42:586b)

Jones, A.R. 1970. Metabolism of trialkyl phosphates. Experientia. 26(5): 492-493.

Jones, A.R. and H. Jackson. 1969. Chemosterilant action of trimethyl phosphate in rodents. Br. J. Pharmacol. 37: 531.

Kenmotsu, K., K. Matsunagal, N. Saito, Y. Ogino and T. Ishida. 1982. Environmental survey of chemicals. 19. Simultaneous determination of organophosphoric acid triesters. Okayama-ken Kankyo Hoken Senta Nenpo. 6: 126-132. (CA 98:95359j)

Kolmark, G. 1956. Mutagenic properties of certain esters of inorganic acids investigated by the Neurospora back mutation test. C.R. Trav. Lab. Carlsberg. 26: 205-220. (Cited in Connor, 1979)

Lee, W.R., S. Abrahamson, R. Valencia, E.S. Von Halle, F.E. Wurgler and S. Zimmering. 1983. The sex linked recessive lethal test for mutagenesis in Drosophila-melanogaster: A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 123(2): 183-279.

Legator, M.S., K.A. Palmer and I.D. Adler. 1973. Collaborative study of in vivo cytogenetic analysis. I. Interpretation of slide preparations. Toxicol. Appl. Pharmacol. 24(3): 337-350.

Lyman, W.J., W.F. Reehl and D.H. Rosenblatt. 1982. Handbook of Chemical Property Estimation Methods. McGraw-Hill Book Co., NY. p. 15-16.

Lyznicki, E.P., Jr., K. Oyama and T.T. Tidwill. 1974. Reactivity of organophosphates. IV. Acid-catalyzed hydrolysis of triethylphosphate: A comparison with ethyl acetate. Can. J. Chem. 52: 1066-1071.

Mabey W. and T. Mill. 1978. Critical review of hydrolysis of organic compounds in water under environmental conditions. J. Phys. Chem. Ref. Data. 7(2): 414.

Machemer, L. and D. Lorke. 1975. Method for testing mutagenic effects of chemicals on spermatogonia of the Chinese hamster. Results obtained with cyclophosphamide, saccharin, and cyclamate. Arzneim.-Forsch. 25(12): 1889-1896.

Midwest Research Institute. 1977. Assessment of the need for the character of and the impact resulting from limitations on aryl phosphates. MRI 4309-L. Kansas City, MO. 279 p.

Moutschen-Dahmen, J., M. Mouschen-Dahmen and N. Degraeve. 1981. Metrifonate and dichlorvos: Cytogenetic investigations. Acta. Pharmacol. Toxicol. 49: 29-39.

NCI (National Cancer Institute). 1978. Bioassay of trimethylphosphate for possible carcinogenicity. NCI Carcinogenesis Tech. Rep. Ser. No. 81. 97 p. [Also publ. as DHEW (NIH) 78-1331]

Newell, G.W., T.A. Jorgenson, C.J. Rushbrook and R. Spanggold. 1976. Preliminary investigation of new orally active reference mutagens. Toxicol. Appl. Pharmacol. 37: 108.

Oishi, H., S. Oishi and K. Hiraga. 1982. Toxicity of several phosphoric acid esters in rats. Toxicol. Lett. 13(1-2): 29-34.

Perry, R.H. and D.W. Green. 1984. Perry's Chemical Engineers' Handbook, 6th ed. McGraw-Hill Book Company, NY. p. 3-62.

Purchase, I.F.H., E. Longstaff, J. Ashby, et al. 1978. An evaluation of 6 short-term tests for detecting organic chemical carcinogens. Br. J. Cancer. 37: 873-948.

Sabljic, A. 1984. Prediction of the nature and strength of soil sorption of organic pollutants by molecular topology. J. Agric. Food. Chem. 32: 243-246.

Schaeppi, U., G. Krinke and W. Kobel. 1984. Prolonged exposure to trimethyl phosphate induces sensory motor neuropathy in the dog. Neurobehav. Toxicol. Teratol. 6(1): 39-50.

Sina, J.F., C.L. Bean, G.R. Dysart, V.I. Taylor and M.O. Bradley. 1983. Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutat. Res. 113(5): 357-391.

Smyth, H.F., Jr., C.P. Carpenter, S. Carrol, U.C. Pozzani, J.A. Striegel and J.S. Nycum. 1969. Range-finding toxicity data. VII. Amer. Ind. Hyg. Assoc. J. 30(5): 470-476.

SRI (SRI Internation). 1984. 1984 Directory of Chemical Producers: United States of America. SRI International, Menlo Park, CA. p. 332.

Van't Hof, J. and L.A. Schairer. 1982. Tradescantia assay system for gaaseous mutagens. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat. Res. 99(3): 303-15.

Veith, G.D., K.J. Macek, S.R. Petrocelli and J. Carroll. 1980. An evaluation of using partition coefficients and water solubility to estimate bioconcentration factors for organic chemicals in fish. Aquatic Tox. 1 ASTM STP. 707: 116-129.

Verschoyle, R.D. and J.R.P. Cabral. 1982. Investigation of the acute toxicity of some trimethyl and triethyl phosphorothioates with particular reference to those causing lung damage. Arch. Toxicol. 51(3): 221-231.

Voogd, C.E., J.A. Jacobs and J.G. Vanderstell. 1972. On the mutagenic activity of dichlorvos. Mutat. Res. 16: 413-416.

Weast, R.C. 1966. CRC Handbook of Chemistry and Physics, 47th ed. The Chemical Rubber Co., Cleveland, OH. p. C-474.

Weber, E., K. Bidwell and M.S. Legator. 1975. Evaluation of the micronuclei test using triethylenemelamine, trimethylphosphate, hycanthone, and niridazole. Mutat. Res. 28(1): 101-106.

Weil, E.D. 1980. Flame retardants (phosphorus compounds). In: Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed., M. Grayson and D. Eckroth, Ed. John Wiley and Sons, Inc., New York. 10: 397, 400-401.

Wolfe, N.L. 1980. Organophosphate and organophosphorothionate esters: Application of linear free energy relationships to estimate hydrolysis rate constants for use in environmental fate assessment. Chemosphere. 9: 571-579.

Wolfenden, R. and R. Williams. 1983. Affinities of phosphoric acids, esters, and amides for solvent water. J. Am. Chem. Soc. 105(4): 1028-1031.

Wyrobek, A.J. and W.R. Bruce. 1975. Chemical induction of sperm abnormalities in mice. Proc. Natl. Acad. Sci. 72: 4425-4429.

Zeiger, E., D.A. Pagano and A.A. Nomeir. 1982. Structure-activity studies on the mutagenicity of tris(2,3-dibromopropyl) phosphate (Tris-BP) and its metabolites in Salmonella. Environ. Mutagen. 4(3): 271-277.

APPENDIX

LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of the following:

CA SEARCH (Files 308, 309, 310, 311, 320
TOXLINE
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP

These searches were conducted in February and March, 1985. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7), and the following secondary sources were reviewed:

ACGIH (American Conference of Governmental Industrial Hygienists). 1980. Documentation of the Threshold Limit Values, 4th ed. (Includes Supplemental Documentation, 1981, 1982, 1983). Cincinnati, OH. 486 p.

ACGIH (American Conference of Governmental Industrial Hygienists). 1983. TLVs: Threshold Limit Values for Chemical Substances and Physical Agents in the Workroom Environment with Intended Changes for 1983-1984. Cincinnati, OH. 94 p.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2A. John Wiley and Sons, NY. 2878 p.

Clayton, G.D. and F.E. Clayton, Ed. 1981. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2B. John Wiley and Sons, NY. p. 2879-3816.

Clayton, G.D. and F.E. Clayton, Ed. 1982. Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2C. John Wiley and Sons, NY. p. 3817-5112.

Grayson, M. and D. Eckroth, Ed. 1978-83. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. John Wiley and Sons, NY. 23 Vol.

Hamilton, A. and H.L. Hardy. 1974. Industrial Toxicology, 3rd ed. Publishing Sciences Group, Inc., MA. 575 p.

IARC (International Agency for Research on Cancer). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. WHO, IARC, Lyons, France.

ITII (International Technology Information Institute). 1982. Toxic and Hazardous Industrial Chemicals Safety Manual for Handling and Disposal with Toxicity and Hazard Data. ITII, Tokyo, Japan. 700 p.

NTP (National Toxicology Program). 1984. Toxicology Research and Testing Program. Chemicals on Standard Protocol. Management Status.

Ouellette, R.P. and J.A. King. 1977. Chemical Week Pesticide Register. McGraw-Hill Book Co., NY.

Sax, I.N. 1979. Dangerous Properties of Industrial Materials, 5th ed. Van Nos Reinhold Co., NY.

SRI (Stanford Research Institute). 1984. Directory of Chemical Producers. Stanford, CA.

U.S. EPA. 1982. Chemical Activities Status Report, 3rd ed. (EPACASR). Offices of Pesticides and Toxic Substances, Washington, DC. EPA 560/TIIS-82-002b.

U.S. EPA. 1983. Status Report on Rebuttable presumption Against Registration (RPAR) or Special Review Process. Registration Standards and the Data Call in Programs. Office of Pesticide Programs, Washington, DC.

U.S. EPA. 1984. CSB Existing Chemical Assessment Tracking System. Name and CAS Number Ordered Indexes. Office of Toxic Substances, Washington, DC.

USITC (United States International Trade Commission). 1983. Synthetic Organic Chemicals. U.S. Production and Sales, 1982, USITC Publication 1422. Washington, DC.

Verschueren, K. 1983. Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Co., NY.

Worthing, C.R. and S.B. Walker, Ed. 1983. The Pesticide Manual. British Crop Protection Council. 695 p.

In addition, the following documents were obtained and reviewed:

FYI-OTS-0784-0328 IN

FYI-OTS-0784-0328 SU

FYI-OTS-0385-0380 Sequence B

In addition, approximately 30 compendia of aquatic toxicity data were reviewed, including the following:

Battelle's Columbus Laboratories. 1971. Water Quality Criteria Data Book, Vol. 3. Effects of Chemicals on Aquatic Life. Selected Data from the Literature through 1968. Prepared for the U.S. EPA under contract No. 68-01-0007. Washington, DC.

Johnson, W.W. and M.T. Finley. 1980. Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates. Summaries of Toxicity Tests Conducted at Columbia National Fisheries Research Laboratory. 1965-1978. U.S. Dept. Interior, Fish and Wildlife Service. Resource Publ. 137. Washington, DC.

McKee, J.E. and H.W. Wolf. 1963. Water Quality Criteria, 2nd ed. Prepared for the Resources Agency of California. State Water Quality Control Board. Publ. No. 3-A.

Pimental, D. 1971. Ecological Effects of Pesticides on Non-Target Species. PB 269605. Prepared for the U.S. EPA, Washington, DC.

Schneider, B.A. 1979. Toxicology Handbook. Mammalian and Aquatic Data. Book 1: Toxicology Data. U.S. EPA, Office Pesticide Programs. Washington, DC. EPA 540/9-79-003. NTIS PB 80-196876.